MOHOGRAFH
OH
BENZOLC ACID
AND
SODIUM BENZOATE

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BENZOIC ACID and SODIUM BENZOATE

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BENZOIC ACID and SODIUM BENZOATE

Summary

The benzoates, both sedium benzoats and benzoic acid, have been investigated extensively for the past hundred years. Essentially, the mechanism for detoxification of benzoates in the body has been known for 50 years. Biochemical work since that time has centered on elaboration of the effects of the benzoates on enzyme systems and on interactions of various drugs and body chemicals with the benzoates.

The absorption of benzoates from the gastrointestinal tract is very rapid and complete (1064, 1168, 1169, 1170). The benzoates are detoxified in the liver by conjugation with glycine resulting in hippuric acid formation (21, 195, 1064, 1474). When insufficient glycine is available benzoyl glucuronide is formed as an alternative (1064, 1474). Elimination is rapid, with 75-100% of the dose appearing in the urine in the first six hours after administration (680, 1064) and the rest within 2-3 days (153). No accumulation of the benzoates or their metabolites has been observed (750).

Glycine depletion caused by benzoate detoxication results in the reduction of creatine (183), glutamine (527), urea (1215, 1319), and uric acid (1064, 1319) formation. The benzoates affect the blood by accelerating autoxidation of oxyhemoglobin (563, 671, 1383, 1463) and myoglobin (662), promoting oxidative disintegration of hemoglobin (563), and shortening prothrombin time (566). The benzoates alter the metabolism of lipids and electrolytes (723). Oxidative phosphorylation is uncoupled from respiration by benzoates in mitochondria (164). The benzoates also inhibit a variety of enzymes (53, 184, 509, 549, 580, 651, 681, 714, 1232).

Simultaneous administration of benzoate and aspirin increases the concentration and persistance of aspirin.in the body (21, 673, 772). Benzoate also increases the effects of procaine, lidocaine, cocaine, tetracaine and dibucaine (902). Under conditions of severe restriction of fluid and salt intake, benzoate increases and prolongs the level of serum penicillin (142, 198, 1444). Sodium bisulphite (1218) and sodium chloride (246) have been found to have synergistic effects with benzoates.

There have been many feeding studies conducted with benzoate sources, that is, benzoic acid or sodium benzoate. Long-term feeding studies have, without exception, used benzoic acid in the dosage regimen. A majority of short-term studies, on the other hand, have used sodium benzoate.

Special studies concerned with the teratogenicity and carcinogenicity of benzoic acid have been conducted within the last several years. Within the past ten years or so work done in Germany, Romania, and the Soviet Union has indicated that previous concepts of the long-term toxicity of the benzoates may have to be reevaluated.

Following is an integrated summation of feeding studies conducted on both sodium benzoate and benzoic acid.

Shtenberg and Ignat'ev ted male and female mice 80 mg of benzoic acid/kg/day for 3 months (1218). At this level, benzoic acid-fed mice showed a significant weight depression (1218). In a long-term feeding study with mice, the same authors found that a dosage level of 40 mg of benzoic acid/kg/day for 17 months resulted in a decreased level of response to physiological stress among test mice (1218). Ignat'ev reported that benzoic acid fed at a level of 80 mg/kg/day for 18 months resulted in a negative effect on body weight and vitality of mice (589). After 18 months on this regimen, the mice had increased liver weights and enlarged spleens, ovaries, and lungs; in addition, the detoxifying capacity of the liver for CC14 was lowered (580).

Fanelli and Halliday found that male rats fed sodium benzoate at 5686 mg/kg/day and female rats fed at 7780 mg/kg/day died of sodium benzoate intoxication within 2 weeks (374). Deuel, et al., reported that rats fed sodium benzoate for 90 days at a dosage of 6290 mg/kg/day exhibited marked toxic effects (317). Kieckebusch and Lang found that sodium benzoate fed at a level of 5000 mg/kg/day resulted in the death of 19 of 28 young test rats within 2 weeks and the remaining 9 by the end of the third week (670).

Griffith fed rats sodium benzoate at 3000 mg/kg/day for 40 days with the result that the test animals showed a distinct growth depression and a 33% mortality attributable to the benzoate (477). Another study at the 3000 mg/kg/day level conducted by Harshbarger showed a 25% mortality of the test rats and obvious toxic effects of sodium benzoate over the 4-5 week duration of feeding (530). Kreis, et al., ted benzoic acid to rats for periods up to 35 days and carried out meticulous histopathological examination of the brain. They found that brain damage occured after 5 days when benzoic acid was fed at a dosage of 3000 mg/kg/day (729).

Four of 5 mature rats died within 4-5 weeks when fed sodium benzoate at a level of 2500 mg/kg/day by Kieckebusch and Lang (670). In contrast to the above study, a feeding study conducted by Deuel, et al., revealed that sodium benzoate at a dosage of 2620 mg/kg/day for 90 days resulted in no visible effects in test rats (317). Fanelli and Halliday corroborated this study by noting no effects in female rats fed 2200 mg of sodium benzoate/kg/day for 28 days; however, male rats in this same experiment showed growth depression at a dosage level of 2000 mg of sodium benzoate/kg/day for 28 days (374).

White found that sodium benzoate fed to rats at a level of 1650 mg/kg/day for 3-6 weeks (4 rats were continued on this regimen for 23 weeks) resulted in marked growth inhibition (1462). Kramer and Tarjan, however, reported no effects from sodium benzoate at a level of 1500 mg/kg/day for 4-8 weeks (725). At a dosage level of 1100 mg/kg/day for 35 days, benzoic acid administered to test rats by Kreis, et al., resulted in retarded growth and impaired food utilization but failed to show any neuropathologic changes (729). Sporn, et al., fed rats benzoic acid at 1 g% (1000 mg/kg)/day for over 11 weeks (1279). Although the benzoic acid at this level depressed the growth rate after 3 weeks, the effect was not readily apparent until after the 11th week (1279). Smyth and Carpenter fed rats sodium benzoate for 30 days at a level of 1090 mg/kg/day and reported that test animals showed no ill effects (1249). Harshbarger likewise observed no evidence of ill effects in rats fed 1000 mg of sodium benzoate/kg/day for 4-5 weeks (530). Marquardt fed rats 750 mg

of benzoic acid/kg/day for 18 months and reported that these animals exhibited decreased food intake and suppressed growth (832).

Kieckebusch and Lang fed benzoic acid at levels of 250 mg/kg/day and 500 mg/kg/day to rats over their entire life-span and continuing over 4 generations of test animals without observing any sign of toxicity (670). One rat fed 500 mg/kg/day lived for 1346 days on the benzoic acid regimen (670). At the 250 mg/kg/day level, life-span of the animals was actually increased (670).

Shtenberg and Ignat'ev fed rats 40 mg/kg/day of benzoic acid for 18 months and then challenged them with a massive dose of 4000 mg of benzoic acid/kg (1218). This resulted in only a 25% mortality, and the workers interpreted this to be indicative of an acquired tolerance to benzoate intoxication through chronic exposure (1218). Ignat'ev reported that in a study conducted on rats over an 18 months period, benzoic acid at a level of 80 mg/kg/day had no effect on body weight, viability, macro or microstructure of parenchymatous organs (589). Furthermore, rats on this regimen survived challenge with a single dose of 4000-5000 mg of benzoic acid/kg (589).

Lucas reported that a dog fed 1000 mg of sodium benzoate/kg/day for 2 days exhibited symptoms of severe intoxication (801). Another dog fed a total dosage of 1430 mg/kg over a 3 day period died (801).

Gerlach reported that 500 mg and 1000 mg of benzoic acid administered daily to humans for 44 consecutive days resulted in no visible effects (443). He also mentioned that 1000 mg of benzoic acid/man/day for 88 days and for 82 days resulted in no unfavorable effects on test subjects (443). Wiley and Bigelow, however, reported that of 12 individuals tested, only 3 could tolerate 35 g of benzoic acid over a 20 day period (1470). Lucas reported also that a single dose of 2000-3000 mg of sodium benzoate in apple cider was toxic for man (801).

In a special study, Minor and Becker injected sodium benzoate at a level of 1000 mg/kg into rats on gestational days 9-11 or 12-14 and found that sodium benzoate exhibited a fetotoxic and teratogenic effect at this level (884). However, no gross anomalies were observed at dosage levels of 100 and 300 mg/kg (884). Dinerman and Ignat'ev reported that Ehrlich ascites carcinoma was more readily implanted in mice receiving benzoic acid in the diet at a level of 300 mg/kg/day for 3 months than in control animals (327).

BENZOIC ACID

Chemical Information

- Nomenclature
 - A. Common Names
 - 1. Benzoic Acid
 - 2. Dracylic Acid
 - B. Chemical Name
 - 1. Benzoic Acid
 - 2. Phenylformic Acid
 - C. Trade Name
 Benzenecarboxylic acid
 - D. Chemical Abstracts Services Unique Registry Number 000065850
- II. Empirical Formula

$$^{\rm C_7H_6O_2}$$
 $^{\rm (C_6H_5COOH)}$

III. Structural Formula



IV. Molecular Weight

122.12

- V. Specifications
 - Α. Chemical 122.0-123.0°C Freezing point 0.005% Residue after Ignition 0.005% Insoluble in Methanol 0.005% Chlorine compounds (as Cl) 0.002% Sulfur compounds (as S) 0.0005% Heavy Metals (as Pb) passes A.C.S test Substances reducing permanganate

B. Food Grade
Appearance and color
Odor

Melting range Ash Heavy metals Benzoic acid Chlorinated compounds Solubility White crystals or needles
No off odor-slight odor
of benzaldehyde
121° to 123°C
0.05% Maximum
20 ppm Maximum
99.5% Minimum
Passes test
Passes test

C. Food Chemicals Codex

Assay

Solidification point
Limits of Impurities
Arsenic
Chlorinated compounds (as CI)
Heavy metals (as Pb)
Readily carbonizable substances
Readily oxidizable substances
Residue on ignition

Not less than 99.5% ${\rm C_7H_6^0_2}$ after drying Between 121 $^{\rm O}$ and 123 $^{\rm O}$

Not more than 3 ppm (0.0003%)
Passes test (about 0.08%)
Not more than 10 ppm (0.001%)
Passes test
Passes test
Not more than 0.05%

VI. Description

A. General Characteristics

Benzoic acid is in the form of white crystals, scales, or needles. It is odorless or has a slightly benzoin- or benzaldehyde-like odor.

В.	Physical Properties	0
	Melting point	122.4 ⁰
	Boiling point:	0
	760 mm Hg	249.2 ⁰ 227 ⁰
	400 mm Hg	227
	200 mm Hg	205.8
	100 mm Hg	186.2 ⁰
	60 mm Hg	172.8

Begins to sublime around 100°

Density 1.321

Volatile with steam

Flash point 121-131°

K at 25° 6.40 x 10°

pH of a saturated solution at 25° 2.8

Vapor pressure 1 mm Hg at 96.0°

Vapor density 4.21

Solubility in water:

At 0°	1.7 g/l
At 10 ⁰	2.1 g/l
At 20 ⁰	2.9 g/l
At 25°	3.4 g/l
At 30°	4.2 g/l
At 40°	6.0 g/l

At 50°	9.5 g/l
A+ 60 ⁰	12.0 g/l
A† 70°	17.7 g/l
A† 80°	27.5 g/l
A† 90 ⁰	45.5 g/l
At 950	68.0 g/I

The solubility in water is increased by alkaline substances such as borax.

Mixtures of excess benzoic acid and water form two liquid phases at 89.7° . The two liquid phases unite at the critical solution temperature of 117.2° . The composition of this critical mixture is 37.34% benzoic acid and 67.66% water.

One gram of benzoic acid dissolves in 2.3 cold alcohol and 1.5 ml boiling alcohol. One gram also dissolves in 4.5 ml chloroform, 3 ml ether, 3 ml acetone, 30 ml carbon tetrachloride, 10 ml benzene, 30 ml carbon disulfide, and in 23 ml oil of turpentine. Benzoic acid is also soluble in volatile and fixed oils. It is slightly soluble in petroleum ether.

C. Stability

Benzoic acid should be stored in well-closed containers in a cool, dry place.

VII. Analytical Methods

There are various methods for the qualitative determination of benzoic acid. Benzoic acid may be crystallized from ether and dissolved in hot water. The precipitate formed when NH₄OH is added, is dissolved in a few milliliters of hot water. Presence of benzoic acid is indicated by the salmon-colored precipitate of ferric benzoate when a few drops of aqueous 0.5% FeCl₃ solution is added.

Another qualitative test for benzoic acid is the modified Mohler test where ${\rm H_2SO_4}$ and ${\rm KNO_3}$ are added to the residue formed from alkaline benzoic acid solution. The mixture is heated, cooled and water is then added to it. When colorless (NH $_4$) $_2$ S solution is added, the red-brown ring formed between layers, indicates benzoic acid. On mixing and heating this solution, benzoic acid can be distinguished from cinnamic acid and salicylic acid. The presence of phenolphthalein interferes (39).

Hoyem developed a method using paper chromatography and ultraviolet spectrophotometry. Paper is spotted and placed in a chromatography tank containing n-butanol, ammonia and water (5:2:3) for 20 hours and exposed to ultraviolet light for development. This procedure can be quantitated by eluting the spots with either 0.01 N HCl or 0.01 N NaOH and measuring at lambda-max on a UV spectrophotometer (578).

Benzoic acid can be quantitated using titrimetry following separation from foods. After purification the residue is dissolved in alcohol and titrated to a phenolphthalein endpoint with NaOH. Vanillin interferes

with this technique (39).

Thin layer chromatography (TLC) has been used to separate and identify preservatives. A TLC system has been reported using polyamide-silaca gels as the mobile phase. Solvent systems such as methanol-water (6:4), Acetone-water (5:5), methanol-ethanol-water (5:1:4), methanol-concentrated NH₃-water 2:1:7, ethanol-water (3:7), and n-Hexane-ethanol (8:2). This method has been used to separate salicylic acid, p-hydroxybenzoic acid, ethyl p-hydroxybenzoate, n-propyl p-hydroxybenzoate, n-butyl p-hydroxybenzoate, benzoic acid, sorbic acid and dehydroacetic acid (931).

Benzoates can be determined in the presence of sorbates using the method of Buglio (212). A column is prepared by packing it with a diatomaceous earth: bicarbonate mixture. The benzoate solution is acidified and extracted with benzene and Na_2SO_4 . The solution is then hydrogenated with a Pd as a catalyst. This step hydrogenates the sorbic acid. The solution is then passed through the column, washed with ether and eluted with 10% glacial acetic acid in ether. The eluent is analyzed on a recording spectrophotometer (from 340 to 250 millimicrons versus a blank). Ninety-nine \pm 0.85% recovery was obtained for benzoate.

Benzoic acid can also be quantitated using spectrophotometry. The sample is extracted with ether, washed with HCl and then NH₄OH. The absorbance of the solution is measured at 272 millimicrons and compared to a previously prepared standard curve. This method is applicable to catsup, other tomatoe products, jams, jellies, beverages containing small amounts of alcohol, soft drinks and fruit juices. This method is not applicable to solids (39).

The above method has been streamlined for the detection of benzoic acid and sorbic acid in fruit beverages. Benzoic acid has a strong absorption maximum at 225 millicrons which is ten times that at 272 millimicrons. A small sample can thus be dissolved in ethyl ether which then is dissolved by adding petroleum ether and sodium sulfate. Usually a beverage will not contain both benzoic and sorbic acids but if such a mixture exists, the two must be separated by thin layer chromatography or gas chromatography. Fumaric acid may interfere at the measuring wavelength but is not usually present in fruit beverages. Since other UV absorbing compounds are present in nonfruit beverages, this method can not be used for them (435). A method for the quantitative determination of benzoic acid using ion exchange chromatography has been developed. The chloride form of De-Acidite FF was used as the resin. The sample is added to centrifuge tubes containing CaCO. After water and ethanol are added, the sample is centrifuged. The supernatant is placed on the prepared column and washed down with a sodium chloride solution in ethanol. The eluant is treated with HCl and ${\rm CUSO}_{\rm A}$ and measured at 220, 230 and 245 millimicrons on a spectrophotometer. method is especially useful for soft drinks, fruit bases and compounds, and gives recoveries of greater than 22% with standard deviations of less than 4% (407).

Polarography has also been used in quantitate benzoic acid. In this method benzoic acid is nitrated and the nitroderivative is analyzed. A sample extract containing 0.1 – 1.0×10^{-3} moles of benzoic acid is treated with H_2SO_4 - HNO_3 and measured polarographically. This method was used for fruit juices and syrups. Standard deviation was 5% with a 6%

distribution at 95% statistical reliability (296).

Semimicro qualitative analysis can be used to identify benzoic acid. When the dry powder is stirred into a small quantity of lead triethylamine, crystalline 4-sided plates are formed singley or in groups. When the powder is stirred into a small quantity of zinc pyridine, hexagonal crystals are formed. If benzoic acid is dissolved in 2% triethanolamine (1:100 to 1:200) and silver nitrate is added, rods or curving blades with irregular ends are developed (39).

Gas chromatography has been used to detect methyl p-hydroxybenzoate and separate it completely from a mixture of sorbic acid, benzoic acid, salicylic acid, dehydroacetic acid, menadione and ethyl, butyl, and propyl p-hydroxybenzoate without any pretreatment. The column was packed with 30% DC 550 silicone on celite 545; the column temperature was 190° and the carrier gas was hydrogen (590).

VIII. Occurrence

Benzoic acid occurs in nature in free and combined forms. As much as 20% may be found in gum benzoin. Among the foods in which benzoic acid occurs naturally are cranberries, prunes, greengage plums, cinnamon and ripe cloves, most berries contain 0.05%. Nearly all vertebrates except birds excrete benzoic acid mainly as hippuric acid.

SODIUM BENZOATE

Chemical Information

- Nomenclature
 - A. Common Names
 - 1. Sodium benzoate
 - 2. Benzoate of soda
 - B. Chemical Name Sodium benzoate
 - C. No Trade Names
 - D. Chemical Abstracts Services Registry Number 000532321
- II. Empirical Formula

 $C_7H_5NaO_2$ ($C_6H\cdot COONa$)

III. Structural Formula





IV. Molecular Weight

144.11

V. Specifications

A. Chemical (U.S.P.)

Description

Solubility

Identification (as Sodium)

(as Benzoic Acid)

Alkalinity (as Sodium hydroxide)

Loss on Drying

Heavy metals (as lead)

Chlorinated compounds

Arsenic

Assay

White, odorless, or nearly odorless, granules, crystalline powder or flakes
1 g dissolves in 2 ml water
Positive
Positive

0.04% Maximum

1.5% Maximum

0.001% Maximum

0.07% Maximum

0.0003% Maximum

99.0% Minimum

Bulk density Screen analysis #8 Mesh minus #20 #80

35-70 fl. oz./lb. 99.0 Minimum 30% Maximum

- B. Food See A above
- C. Food Chemicals Codex

Assay

Not less than 99% C₇H_ENaO₂, calculated on the dried basis

Limits of Impurities Alkalinity (as NaOH) Arsenic (as As) Chlorinated compounds (as CI) Passes test (about 0.07%) Heavy metals (as Pb) Loss on drying

Not more than 0.04% Not more than 3 ppm (0.0003%) Not more than 10 ppm (0.001%) Not more than 1.5%

VI. Description

A. General Characteristics

Sodium benzoate occurs as white, odorless or nearly odorless, granules, crystalline powder or flakes. It has a sweetish, astringent taste without bitterness.

B. Physical Properties

At 0° C, 62.8 g dissolve in 100 ml water; at 20° C, 66.0 g dissolve in 100 ml and 74.2 g dissolve in 100 ml water at 100° C. In 100 ml ethyl alcohol, 0.81 gm dissolve at 0° C; 1.64 gm dissolve at 25° C and 8.30 gm dissolve at 78°C. One gram dissolves in 50 ml of 90% alcohol.

An aqueous solution of benzoic acid is alkaline to litmus and has a pH of around pH 8.

VII. Analytical Methods

See Benzoic Acid

VIII. Occurrence

Sodium benzoate does not occur naturally. However, benzoic acid from which it is derived occurs naturally in berries.

BENZOIC ACID

Biological Data

I. Acute Toxicity

Substance	Animal	Route	Dosage (mg/kg)	Measurement	Ref.
Benzoic Acid	Rat	1.٧.	1590-1838	LD ₅₀	508
Benzoic Acid	Rat	P.O.	2000-2500	LD50	589
Benzoic Acid	Guinea Pig	I.P.	1400	LD50	508
Benzoic Acid	Rabbi t	P.O.	2000	LD50 LD100 LD100	508
Benzoic Acid	Rabbit (Fasting)	P.O.	1520-1830	LD 100	508
Benzoic Acid	Rabbit (Dry-Fed)	P.O.	1700	LD 100 LD 100 LD 100 LD 100	508
Benzoic Acid	Cat	P.O.	2000	LD 100	508
Benzoic Acid	Dog	P.O.	2000	LD 100 100	508

One sheep was given 79.7 gm of benzoic acid in 24 hours by rumen infusion. This animal refused food offered six hours after the infusion began. Severe muscular weakness accompanied by muscular tremors developed on the second day after infusion. The sheep died on the third day (834).

Another sheep was given 9.6, 22.4, and 54.0 gm of benzoic acid in 24 hours by abomasal infusion. No symptoms were observed. However, when 88 gm of benzoic acid were infused over 24 hours, the sheep died during the next 24 hours (834).

The death of one sheep three days after a dosage of benzoic acid at 1100 mg/kg and of another sheep one day after a dosage at 1800 mg/kg indicates that 1000 mg/kg is close to a lethal dose for sheep (834).

II. Short-Term Studies

Mice

Groups of 50 male and 50 female mice weighing 8-10 g and housed in groups of 10-25 received, by oral intubation, 0 or 80 mg/kg/day of benzoic acid. Feed and water were provided ad lib. The mice were observed for general condition and behavior and survival. Food consumption and weight gain were recorded daily. Tests were carried out to determine the effects of hunger, physical stress, and poisoning with carbon tetrachloride on the test animals compared with untreated controls. The feeding regimen was continued for three months (1218).

At the termination of the feeding regimen, surviving mice were subjected to a restricted dietary intake (reduced 90%) for up to five days. The mice received water ad lib, and the dosing regimen with benzoic acid was continued by oral intubation. Weight loss and mortality rate were recorded (1218).

Test animals grew significantly less than controls; although, this was not related to food intake. In fact, the average daily food intake for test mice was 2.57 and 2.65 respectively for males and females compared to 2.33 and 2.81 respectively for male and female controls. Female test mice had a daily food intake of 94% of that of the controls and a daily water intake of 85% of controls. Weight gain was 71% of controls. Male control animals, on the other hand, had a food intake of 90% of test mice and a water intake of 93% of test mice. In spite of this, male test mice showed a weight gain of only 66% of control mice (1218).

Rats

In a rather long discussion of toxicity criteria, Shporn, et al., mentioned a feeding study conducted on rats in which benzoic acid was fed to the animals receiving a stock diet containing 18% casein:

				Growth Stir	nulation
<u>Substance</u>	No. of Animals	Diet	_Concentration	Apparent	Real
Benzoic Acid	12	18% Casein	1 g%	11 wks.	3 wks.
Benzoic Acid	12	18% Casein	5 g %	7 wks.	2 wks.
Benzoic Acid	12	18% Casein	10 g%	3 wks.	1 wk.

The apparent stimulation means that the actual weight of the test animals is significantly higher than that of controls; whereas, the real stimulation means that the growth rate/animal is higher. The point is that a compound may have a deleterious effect (as in this case) despite an apparent stimulation in growth (1279).

The term 1 g% is apparently 1 gm/100 ml of benzoic acid solution. At this level, benzoic acid affects growth negatively after 3 weeks, but this does not become apparent until after the 11th week (1279).

Groups of growing male Royal Wistar rats, housed individually, were divided into groups and fed various regimens of benzoic acid (see table). Benzoic acid was incorporated in the diet, and food and water were provided ad lib (729).

			<u>Benzoic</u>	Acid	
Group		No. of Animals	% in Food	Days of Admin.	Test Duration
Α	1	5	3	1	1
	11	5	3	2	2
	$\Pi\Pi$	5	3	3	3
	IV	8	5	5	5
В	1	10	3	5	: 5
-	11	15	3	5	24 or 35
С	ļ	5	1.1	7	. 7
	H	8	1 1	14	14
	+111	10	1.1	35	35
Control	ŀ	5	0		3
Control	11	7	0	•	35

The three series (A,B,C) were set-up to determine the time after which organic damages first occur. Since 50% of the test animals given 3% benzoic acid died by the fifth day, this interval was taken to be the time at which all animals suffered damages. Animals surviving the fifth day in group B (series II) were put on a benzoic acid-free stock diet until the 24th or 35th day in order to determine damages extending beyond the duration of administration (729).

Animals were killed by decapitation and immediately dissected. Histological examination of heart, liver, and kidneys was carried out. After careful preparation, the brain was removed, imbedded in paraffin, sliced, and stained with haematoxylin-eosin or cresol violet (Kresylviolett). The brain was carefully examined for micropathologic change (729).

At the 3% benzoic acid dosage level, most animals would show abnormal behavior after 4-5 days. Restlessness, aggressiveness, tonic-clonic cramps, and fear reactions were characteristic. After 5 days, half the animals had died (729).

Brain damage was demonstrable histologically at the 3% benzoic acid level and consisted of necrosis of parenchymal cells of the stratum granulosum of the fascia dentata and of the cortex of the lobus piriformis. These changes occurred consistently after 5 days on the 3% level and occasionally after 3 days. Other organs, however, did not appear to be affected. The brain damage was still evident 19 or 30 days after benzoic acid feeding ceased (729).

At the 1.1% level for 35 days, benzoic acid retarded growth and impaired food utilization, but failed to show any neurotoxic signs of pathological changes in the brain (729).

Man

In experiments on human volunteers (numbers not given), including himself, Gerlach reported the effects of benzoic acid. The intake of 10 g of benzoic acid orally resulted in absolutely no effect on body temperature, pulse, respiration, digestion, or any other untoward effects. Benzoic acid administered at the rate of 0.5 g and 1.0 g (daily) for 44 consecutive days showed no visible effects; body weight of these individuals remained stable (443).

Eighty-two daily doses of 1 g benzoic acid over a period of 86 days or 88 daily doses of 1 g benzoic acid over a period of 92 days resulted in no recognizable unfavorable effects. Gerlach reports that a daily intake of 1 g benzoic acid for 6 consecutive days had no effect on serum albumin or on the utilization of nitrates and lipid components of foods (443).

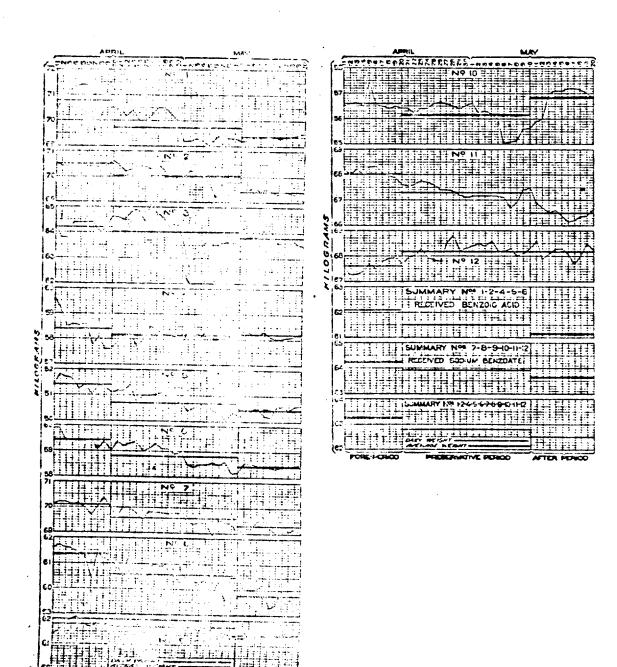
A regimen was designed in which 12 individuals would be given benzoic acid orally as follows for 20 consecutive days: 1 g for 5 days, 1.5 g for 5 days, 2 g for 5 days, and 2.5 g for 5 days. Total dosage would be 35 g of benzoic acid over a 20 day period (1470).

Following are tables (II and III) showing the schedule of administration and average body weights for test subjects: (1470)

TABLE II

-Schedule of administration of preservative (to expenses)

to grand determine	70 Va.L	70	64 200	5 3		5'4 No. 0.	64	ec.	h	te (ση <u>S.C.</u> No. ω.	<u>; 7</u>	68
Treed got most at: Alpha	finit.	(Ime	G 25.	ØMi	Gr.r.	Gus.	Gms.	Gr.v. 0.6 1.6 1.0	1.0	1.0 1.0	1.0 1.0	Gms. 20 10 10 10
Tellin forte	3	5	5	, S	5	5	4, f	4.6	≱ ę Santas	4.9	49	
Beach Letterfield Appail Letter Classification 19 Letter 20 Bit 1900	1.5	1.5	1.5	1.5	1.5	1.3	1.5 1.5 1.5 1.5	1.5	1.5 1.5 1.5 1.5	1.5 1.5 1.5 1.5	1.5 1.5	L & 1. & L & L & L &
Test twenty) -		7.5	1.5	3,	•	5.1	5.1	7.5	7.5	7.5	7.5	7.8
The first victory on the second of the secon		e161616161		ř 2 2 3	2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2	2000000	20.7020	1313151313		2 2 2 4 2	222222	2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2
Total (la li- vidual	. 16	19	1	19.0		10	11	10	19	, A	1.)	10
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Total perindi- vidual		<u> </u>	6	12.5	3		, (,:	12.	0	0	2.5	
found per in his based a ren- tin preserve alive pariod.	•) 24.0	12.	35. 0	, ,		. 25 1	36.1	22.	· 24	24.9	=



Average body weights

Of the 12 individuals tested, only 3 took the entire dosage of 35 g. Benzoic acid produced marked symptoms of discomfort and malaise. The most common symptoms were nausea and headache; although, weakness, burning and irritation of the esophagus, hunger and indigestion were also experienced (1470).

III. Long-Term Studies

Mi ce

Groups of 25 male and 25 female mice, weighing 10-15 g, were given 0 and 40 mg of benzoic acid/kg/day for 17 months. Benzoic acid was fed as a paste prior to the main feed, and food and water were provided ad lib. The mice were observed for general condition, behavior, and survival. Food consumption and weight gain were recorded daily. Further testing was carried out to determine the effects of hunger on test animals. At the completion of the study, organ weights were recorded (1218).

After 17 months, survivors were subjected to fasting for 5 days, during which time water was provided ad lib, and the benzoic acid regimen was continued by oral intubation. Weight loss and mortality weight were recorded. Survivors at this time were fitted with a weight on their tail and left to swim in a water bath. The time until they sank of exhaustion was recorded (1218).

Mice fed benzoic acid had a higher mortality than did control animals after the final fasting period (50% to 12%). The weight loss of the benzoic acid-treated animals was 26% during fasting but only 17% in control animals. In addition, control animals regained lost weight almost twice as quickly as benzoic acid-dosed animals (1218).

Data were omitted to a major extent, on the long-term mouse experiment. Despite extensive testing regimen reported, there were no data provided on food consumption or on weight gain/loss as compared to controls. The major emphasis reported was the effect of stress on mice fed benzoic acid, and the results in effect, were that mice fed benzoic acid at the level of 40 mg/kg/day for 17 months responded to physiological stress to a much lesser degree than did controls.

Groups of mice were fed 0, 40, and 80 mg/kg/day of benzoic acid for periods of 3, 8, and 18 months. The following observations/determinations were made: observation of general condition, production of offspring, viability under experimental conditions, viability under stress, utilization of food and water, weight gain, body cooling tests, physical stress tests, CC14 and K2HPO4 tests, blood determinations, complement titer, leucocytic phagocytic activity, blood oxidase activity, blood alkalinity, blood ketone level, protein in urine, carcinogenicity, and histopathological observations (589).

Following are data on body weight, food and water uptake, viability and endurance of physical load in the 18 month study with mice (589).

Mice (80 mg/kg/day) Body Weight, Food and Water Uptake, Viability

	Absolute Gain		Relative Gain	Upt	Viability		
	<u>In g</u>	In %	<u>In %</u>	Food (g)	Water (ml)	Exp.	Fasti
Control Males	16.57	199.39	109.7	2.33	3.67	60	3.86
Control Females	12.13	166.83	93.26	2.81	4.14	72	5.22
Benzoic Acid							
Males	11.28	124.52	80.87	2.57	3.85	68	4.4
Benzoic Acid							
Females	10.95	118.23	78.05	2.65	3.52	68	4.4

Mice (40 mg/kg/day) Endurance of Physical Load

Swimming Time in Minutes	Added Weight/To	tal Body Weight
er en	5%	<u>50%</u>
Control Males	4.66	5.0
Control Females	5.17	5.5
Benzoic Acid Males	11.71	
Benzoic Acid Females	16.33	ve an

Overall, benzoic acid exerted a negative effect on mice. Body weight and viability were negatively affected. Carcinogenic effects attributable to benzoic acid were noted. The detoxifying capacity of the liver for CC14 was lowered. After 18 months, mice examined had increased liver weights and enlarged spleens, ovaries, and lungs (589).

This study was reported in several other papers, none of which provided data sufficient to justify the conclusions reached. That is not to say, however, the justification does not exist. It simply is not reported.

Rats

Groups of 10 male and 10 female rats weighing 100-120 g, were fed 40 mg of benzoic acid/kg/day for 18 months. An undisclosed number of controls were provided. The benzoic acid was fed in a paste prior to the main feed; food and water were provided ad lib (1218).

Food and water consumption, weight gain, and effects of stress factors (fasting) were recorded. Blood determinations included: serum complement titer, phagocytic activity of leucocytes, serum ceruloplasmin, blood alkalinity, blood ketones, blood morphology, erythrocyte sedimentation rate, and C-reactive serum protein (1218).

After 18 months, surviving animals were fasted for 13 days, during which time benzoic acid regimen was continued by oral intubation, and water was provided ad lib. Weight loss, mortality rate, and rate of weight-recovery were recorded (1218).

Rats fed benzoic acid for 18 months developed tolerance to a lethal dose of the compound given terminally. In rats fed benzoic acid at the level of 40 mg/kg/day for 18 months, a massive dose of 4000 mg/kg of sodium benzoate resulted in only a 25% mortality; whereas, a dose of 3600 mg/kg of sodium benzoate resulted in a 100% mortality of control animals (1218).

Data were not reported for the most part. From the data included one can determine that the average daily food and water intake of male test rats was significantly lower than that of controls. Female test animals and controls differed little.

Groups of rats were fed 0, 40, and 80 mg/kg/day of benzoic acid for periods of 3, 8, and 18 months. The following observations/determinations were made. Observation of general condition, production of offspring, viability under experimental conditions, viability under stress, utilization of food and water, weight gain, body cooling and physical stress CCI4 and K2HP04 tests: blood determination, complement titer, leucocytic phagocytic activity, blood oxidase activity, blood alkalinity,

blood ketone level, protein in urine, carcinogenicity, and histopathological observations (589).

There seemed to be no effect on body weight and viability of rats over an 18 month period on a dosage regimen of benzoic acid at 80 mg/kg/day. No changes in either macro or microstructure of parenchymatous organs were noted in test rats. Rats fed benzoic acid at a dosage of 80 mg/kg/day for 18 months survived a challenge with a massive overdose of benzoic acid up to 4000-5000 mg/kg (589).

Seventy-five 5 week-old Wistar rats weighing 50-60 g, housed individually were separated into 2 groups and provided food and water ad lib. One group of 50 (20 female, 30 male) rats was fed benzoic acid at 1.5% level in their diet. The other group of 25 (12 female, 13 male) rats served as control (832).

During the first 5 months, animals were weighed weekly. After that period, weight determinations were made every 4 weeks. The experiment was continuing at the time of the report (18 months) (832).

The difference between the control animals and the animals fed 1.5% benzoate was an average of 10 g/animal at 60 days, 18 g/animal at 340 days, and 15 g/animal at 430 days. In all cases, animals fed 1.5% benzoic acid had a decreased food intake and suppressed growth (832).

In another experiment, Marquardt fed 20 male Wistar rats and 20 male Osborne-Mendel rats benzoic acid at a 1.5% level. Ten male Wistar and 10 male Osborne-Mendel rats were used as controls. Results were similar (824).

At a 1.5% level, benzoic acid is toxic (832).

Three groups of 40 (20 male, 20 female) Bayer-Elberfeld rats housed in pairs were fed benzoic acid at 0, 0.5, and 1.0% levels in the diet. For the first 8 weeks, a paired feeding technique was used, and after 8 weeks, food was provided ad lib. Water was provided ad lib at all times (670).

For the first 8 weeks, the animals were weighed weekly; after the first 8 weeks, the rats were weighed every 4 weeks. At the age of 11-12 weeks, males and females were paired and housed together for 14 days. If pregnancy did not result, this pairing was repeated 8 weeks later. A final mating took place on the 48th week. The offspring of these were carefully observed over 2 generations and were fed benzoic acid of 4 generations (670).

Protein efficiency (weight gain/g food protein) was calculated, Litter size (number and weight) was recorded and histological determinations were made on 4 generations (670).

No signs of toxicity appeared at either the 0.5% or at the 1.0% dosage level over an entire life span of the rat and over 4 generations. Benzoic acid at the 0.5% level actually increased the life-span significantly; some animals lived over 1000 days. One survived 1346 days on a 1% benzoic acid diet (670).

IV. Special Studies

Carcinogenic

One hundred and thirty-nine mice were divided into a test group of 90 and a control group of 49. All animals received a stock diet, the test animals having a .2% level of benzoic acid as a supplement. For three months the mice received benzoic acid or stock diet. This was followed by intraperitoneal inoculation with Ehrlich ascites carcinoma (1-1.25 x 106 cells) (327).

Mice were weighed before injection and their abdominal volume measured. Measurements were made at intervals of four days from day of transplantation until death. The observation period was 53-66 days, after which time all surviving mice were sacrificed and autopsied (327).

The following table shows tumor incidence:

Substance	No. of Mice	Observation Period (Days) No.	No. of Tumors 5
Benzoic Acid	90	53	62	60.8
Control	49	· 66	16	32.6

Successful inoculation of tumors was more frequent in benzoic acid fed mice. In addition to this, carcinoma development was also more intensive and the survival time of these mice was the shortest (327).

SODIUM BENZOATE

Biological Data

Acute Toxicity

Substance	Animal	Sex & No.	Route	Dosage (mg/kg)	Meas.	Ref.
Sodium Benzoate	Rat (USC)	70 (M & F)	P.0.	2100	LD _F	317
Sodium Benzoate	Rat (Sherman)	M & F	P.O.	3450	LD50	317
Sodium Benzoate	Rat (Sherman)	10 M, 10 F	P.O.	4070	LD ₅₀ LD ₅₀ LD ₅₀	1249

A negro male with marked yellow atrophy of the liver had a severe reaction on two occasions, approximately four hours after ingesting sodium benzoate, manifested by excruciating substernal pain, elevation of blood pressure, dyspnea, and orthopnea followed by shock. Three days after ingesting the second dose of sodium benzoate, the patient developed signs of systemic infection. The white blood count showed an absence of neutrophils, but a normal to increased number of basophils and eosinophils. The bone marrow showed arrest of maturation of the neutrophyllic myeloid elements, but normal maturation of eosinophils and basophils. There was later a marked leucocytic response to a generalized septicemia; this response was indicative of a recovery of the bone marrow. The patient died two months after the first challenge with sodium benzoate of liver failure (681).

II. Short-Term Studies

Rats

Young male white rats were fed diets containing 0, 1.5, 2.0, 2.5, and 3.0% sodium benzoate. The increase in the weight of the rats receiving 1.5, 2.0, and 2.5% sodium benzoate diets was comparable to that of the control group. However, animals at the 3.0% level showed a distinct growth depression even though their food consumption was comparable to control. At this level, one-third of the rats died (477).

White fed a low-casein diet as a basal ration to rats and studied the effects of various compounds on animals receiving sodium benzoate. Weanling male rats, housed individually, were fed a low-casein diet ad lib. Each animal received a daily supplement of 400 mg of dried yeast. When the rats reached a body weight of 75-85 g, sodium benzoate was added as 5% of the diet, a dosage level previously found to be the minimum quantity necessary to produce marked growth inhibition. This regimen was continued for 3-6 weeks (1462).

The average daily weight gain of 115 control animals was 1.3 g/rat, and the average daily food consumption was 6.2 g/rat. The corresponding figures for 106 test animals were 0.0 g/rat for weight gain and 3.3 g/rat for food intake. The addition of sodium benzoate at the 5% level produced the desired stunting without any obvious signs of gross toxicity. Four rats were used as long-time controls and were permitted to ingest sodium benzoate at the 5% level for 23 weeks. During this time, no gross toxicity other than growth depression, was observed among all members, of the group; however, a rat was occasionally seen which could not tolerate

sodium benzoate at this level, data from these rats were not used (1462).

Rats fed 1947-2195 mg of sodium benzoate/kg/day for 3-6 weeks (and up to 23 weeks) exhibited a severe depression of the growth rate (1462).

Harshbarger fed 4 week-old white rats 0, 1, and 3% sodium benzoate for 4-5 weeks. A paired feeding technique was used with a modification so that a control animal was compared with 2 test animals. Eight triplicates (24 rats) were fed sodium benzoate at varying levels. In each triplicate, litter mates of the same sex were used. Sodium benzoate was administered as a paste in the diet, and water was provided ad lib (530).

In the first study, control animals were fed calcium lactate and test control diet was compared with 1% sodium benzoate. In the second study, this control diet was compared with 1% sodium benzoate. In the final study conducted by Harshberger, animals from the second study, including the control animals, were fed 3% sodium benzoate (530).

In the first study, sodium benzoate at the 3% level proved toxic. The amount of weight gain was much less than that of control, and 2 of 8 rats died before the test period was ended. In the second study, sodium benzoate at the 1% level had no effect on weight gain. In the final study, 3% sodium benzoate in the diet resulted in a more toxic effect for control animals that had not previously encountered it than it did for those animals encountering it previously at the 1% and 3% levels. This seems to be indicative of a developed resistance by rats to the toxic effect of sodium benzoate (530).

Deaths caused by sodium benzoate occurred 7 to 20 days after initiation of dosage. Animals living longer than 20 days exhibited an increasing resistance to its toxic effects (530).

In another short-term study, 10 Sherman rats (5 male, 5 female) were housed in groups of 5 of each sex at each dosage level and fed sodium benzoate at levels of 0, 16, and other levels (3) up to 1090 mg/kg/day for a period of 30 days. Food and water were provided ad lib. The diet for each cage was kept in a separate jar and was weighed before and again with the uneaten food after the 30 day test period (1249).

Increase in body weight (compared to controls), reduction in appetite, death, and micropathology of adrenals, upper intestine, kidney, liver, and spleen were noted (1249).

At a level of 1090 mg of sodium benzoate/kg/day for a period of 30 days, there were no toxic effects observed (1249).

Five groups of 10 young Sherman rats (5 male, 5 female) were fed sodium benzoate at the following percentages of the diet: 0, 1, 2, 4, and 8%. After 90 days, animals were sacrificed, and their tissues were examined for micropathologic changes. The following table shows the results (317):

The results of the 90-day feeding tests on rats receiving diets containing different levels of sodium benzoate or a sodium benzoate-tree control diet

Table IV

Category	Bodium binteste- tree control					
	U.C.	±.0	: 2.0	4.0	6.0	
Sodium benzonte dosnge, g./kg./dep	(0.9)	6.54	1.22	2.62	6.29	
Diet consumed, p. ret/coy	11.97	0.60	12.63	12.30	21.98	
p for t-test		0.54	0.62	0.75	0.99	
Average weight gain of cortiving rate, g		170.7	160.5	150.0	100.2	
p for t—test	• •••	6.58	0.86	0,50	0.06	
Liver weight, So body weight		3.03	2.97	2.12	4.46*	
p for t—test		0.25	0.26	9.59	0.0003	
Ridney weight, Se body weight		9.6-2	6.65	e.70	0.90*	
p for t-tect		1.00	0.65	0.06	0.0001	
Uninfected rats		9	10	8	8	
Toxic deaths		-0	, e	0	4	
Average number days to death		••••			13.0	
Sets of tissues examined from uninfected rats.		9	10	8	7	
Sets with major pathology		0	0	0	1	
Sets with any rathology.	0	1	- 1	1	7	

^{*}Results statistically rignificant as compaced with control

At the 8% level in the diet, sodium benzoate depressed the growth rate of surviving rats by one-third, though food consumption remained the same as that of control animals. The weights of both liver and kidneys of these rats were significantly higher than those of controls. Frequent pathologic lesions of the liver and kidneys were evident (317).

Sodium benzoate fed at the 4% level (and lower) seemed to have no harmful effect on the rats over a period of 90 days. Rats consumed, at the 4% level, sodium benzoate amounting to 2620 mg/kg/day for 90 days (317).

Thirty-six Sherman rats (18 male, 18 female), 23 days-old, were assigned randomly to 6 cages so that there were 6 male and 6 female rats at the following dosage levels of sodium benzoate: 0, 2 and 5%. The rats were given a basal diet and water ad lib for three days, after which they received their respective sodium benzoate diets for 28 days (317).

Initially, body weights were recorded, and animals were observed for any gross abnormalities. During the testing regimen, the rats were weighed individually twice a week and were inspected daily for signs of toxic response. Food consumption for each group was recorded weekly, and the daily intake of sodium benzoate was calculated using the average body weights for each group (374).

All animals receiving 5% sodium benzoate died during the first or second week after exhibiting hyperexcitability, urinary incontinence, and convulsive seizures. Apart from a slight weight depression significant for male rats, animals at the 2% level were similar to controls (374).

Sodium benzoate at levels of 5686 mg/kg/day for males and 7780 mg/kg/day for females was lethal within 14 days. At dosages of 2000 mg/kg/day for males and 2200 mg/kg/day for females, sodium benzoate showed a slight growth depression for males and no visible effect for females over a period of 28 days (374).

Twenty-eight young rats were fed sodium benzoate as a 5% component of the diet. Within 2 weeks, 19 animals died of benzoate poisoning. The remaining 9 rats died by the end of the 3rd week. A similar feeding test involving 5 adult rats resulted in the death of 4 of them within 4-5 weeks (670).

Two groups of 10 male 8 week-old Wistar rats were fed a stock diet, and the test group was fed sodium benzoate as a 1.5% component (added as a 10% aqueous solution). After 4 weeks, a paprika puree containing 45 Ug of carotene per animal per day was added to the stock diet. After 6 weeks, half of the animals were killed and the remainder were killed after 8 weeks (725).

Growth was recorded and kidney and liver vitamin A was determined (725).

There appeared to be no significant effects of sodium benzoate at the 1.5% level. Vitamin A content of liver and kidneys was not significantly different from that of controls (725).

In another series, Kramer and Tarjan reported that sodium benzoate at the 3% level depressed growth. This effect was based on results of a period of adapting rats to a benzoate diet (725). Unfortunately, data provided were not sufficient to enable one to make a meaningful determination of the benzoate dosage at a mg/kg/day figure.

This report corroborates in essence the reports of Kieckebusch and Lang, Marquardt, and of Kreis, et al., concerning the effects of benzoic acid. That is, that at a level of approxmiately 1%, the benzoates are at maximum non-toxic level; higher than this, they result in decreased food intake, depressed growth, and toxic effects on test animals.

Dog

A dog weighing 3.5 kg was fasted for 24 hours and then given 1 g of sodium benzoate. In 30 minutes, the animal showed evidence of muscular weakness and nausea, lay quietly, and breathed in a laborious manner. This behavior continued for 6 hours. On the following day, the animal was given 4 g of sodium benzoate. The dog became very weak within an hour; breathing was labored; and, after several hard convulsions, the animal died 2 hours and 20 minutes after administration of the sodium benzoate. Therefore, a dosage of 1430 mg of sodium benzoate/kg over a 2 day period resulted in the death of the dog (801).

Two dogs were fed on dog biscuits and water for several weeks then fasted for 36 hours. One dog weighing 3.5 kg was fed 3.5 g of sodium benzoate (citric acid + hydrochloric acid added to convert the sodium salt into the acid form), the other dog weighing 4.25 kg was fed a control diet plus the same amount of citric acid + hydrochloric acid. The sodium benzoate-fed dog showed great muscular weakness and tremor after an hour; the control dog was unaffected. The same regimen was

repeated the second day, and the results were similar. The dog receiving 1000 mg of sodium benzoate/kg/day for 2 days exhibited prostration, stiffness of the muscles, and internal bleeding (801).

Man

Twenty-four individuals were observed in an experiment in which 12 received pure apple juice and 12 received juice containing 0.1% sodium benzoate. In comparison with those who received pure apple juice, men who drank the apple juice containing 0.1% sodium benzoate exhibited the following symptoms: burning taste, fullness in the head, headache, nervousness, nausea, vomiting, itching of the skin, unusual perspiration, constipation, decreased flow of urine, increased specific gravity of urine, and albuminuria. A liter of filtered cider containing 0.2-0.3% sodium benzoate (2-3 g) caused albuminuria within 3 hours in the largest and soundest individuals (801).

Lucas reports, however, that he has ingested as much as 6 g/day for 3 successive days without the slightest discomfort (801).

III. Long-Term Studies

None

IV. Special Studies

<u>Teratogenic</u>

Sprague-Dawley rats were injected intraperitoneally on gestation day 9-11 or 12-14 with 90 and 600 mk/gk sodium chloride as a control. Sodium benzoate at levels of 100, 315, and 1000 mg/kg was injected in the test animals. Fetal body weight was reduced from 5.25 g in control group to 4.56 g in test group dosed at 1000 mg/kg sodium benzoate. At this level, in utero deaths were increased by 12% and gross anomalies were observed (884).

BENZOIC ACID and SODIUM BENZOATE

Biochemical Aspects

1. Breakdown

No information available from sources obtained.

II. Absorption-Distribution

The absorption of sodium benzoate or benzoic acid from the gastrointestinal tract appears to be vary rapid and complete (1064, 1168, 1169, 1170). The method of absorption is simple diffusion of the unionized molecules (1164, 1169, 1170).

III. Metabolism and Excretion

In an extensive study, Bridges et al. determined the metabolites, and their rates of excretion, of sodium benzoate in man and twenty other species of animals (195). The data obtained is presented in Table V (195).

Table V Metabolites of [14 Changole acid in arine in various species

Solium J*CDent vite was administered orally as described in the text. The direct 2th urine (unless otherwise stated) of each animal was chromatographed on Whatman no. I paper usee Table I for solvents) and the paper secaled in a Packer I radio chromatogram seanner. Where three or more animals were used the results are expressed as averages with ranges in parentheses. Where only one or two animals of a species were used the individual results are given. The amounts of the metabolites were estimated from the chromatogram seans as described in the text. M. male: F. female.

•	Family or other	Dose		140	% of 24 h excretion found				
9		Bengoic acid (mg kg)	eq: (p() animal)	"C exercted in 24 h (% of dose)	Benzoic acid	Hippuric acid	Benzoyl glucuronide	Ornithuric	
Species (no. and sex)	description	(m) v3:	the courses	i. 9 m morel	****		Entratomar		
Primates			•	99.4, 99.7	Û	100	0	0	
Man (2M)	Homo		3.8		0	100	0	ŏ	
Rhesus monkey (3F)	Macaca	20	6.8	47 (33-59)		51, 53	-	ŏ	
Squirrel monkey (2F)	Saimiri	30	5.5	16, 19	14, 18	100	5, Trace Trace	0	
Capuchin (1F)	Ceòns	50	8.5	57	. U	1110	Irace	v	
Artiodactyla							_	_	
Pig (large white) (2F)	Suilae	50	10.1	49, 51	15, 7	83, 93	Trace	0	
Lagomorpha									
Rabbit (New Zealand White)	Leporidae	49	.8.4	60 (36-77)	0	100	U	0	
(3F)		200	9.7	86 (79-95)	Trace	98 (97-100)	2 (0-5)	0	
Rodents									
Rat (Wistar albino) (3F)	Muridae	30	1.0	100 (95-102)	1 (Trace-2)	99 (97-190)	Trace	0	
Mouse (I.C.I.) (3 × 10F)*	Muridae	36	0.6	35 (43-67)	Trace	95 (94-96)	5 (4-6)	0	
Guinea pig (English) (3F)	Cariidae	49	3.0	79 (63-94)	Trace	:rs (97-99)	3 (2-3)	0	
Hamster (golden) (3F)	Cricetidae	52	2.1	99 (98-101)	1	97 (97-98)	1 (1-2)	0	
	Cricetidae	56	2.5	28 (24-102)	Trace	160 (99-100)	0	ė	
Lemming (steppe) (3F)	Cricilidae	29	3.3	73 (66-82)	2 (0-4)	98 (96-100)	ō	Ó	
Gerbil (3F)	Crictions	••			* (* -,	,	-	-	
Carnivors			9.7	29, 86	Trace	100	0	0	
Cat (mongrel) (2F)	Felidae	51		94 (87-199)	0	82 (71-100)	18 (Trace-25)		
Dog (mongrel) (2F, 1M)	Canidae	51	8.2 7.1	69 (37-90)	9 (9-12)	70 (67-72)	22 (21-22)	ŏ	
Ferret (mongrel) (3F)	Mustelidae	50			9 (3-12)	47 (40-54)	44 (41-49)	ŏ	
		198	7.5	75 (62-88)		30 (24-37)	49 (42-53)	ŏ	
		400	7.5	67 (38-74)	22 (21-23)	30 (24-31)	45 (42-99)	v	
Insectivora					5, 7	76, 86	19, 7	0	
Hedgehog (European) (2M)	Erinaceidae ,	50	6.4	67, 78	ο, ,	10, 80	19, /	U	
Chiroptera						_		_	
Fruit bat (Indian) (2F)	Pteropidae	50	7.8	49, 31	12, 30	Trace	88, 70	0	
Birds		1							
Chicken (Light Sussex (5F)	Phasianidae	50	9.3	56 (38-69)	22 (16-28)	21 (10-43)	3 (0-8)	54 (40-69)	
Pigeon (3F)	Columbidae	50	6.3	87 (84-102)	15 (13-20)	64 (80-87)	i (Trace-3)	0	
Reptiles									
Turtle (side-necked) (1)*	See footnote	50	6.2	395	10	16	2	72	
Gecko (1 × 3F);	Gekkonidae	19	2.5	321	3	4	6	85	

The urines of ten animals were pooled for each determination.
The urines of three animals were pooled.

[†] Also called hidden necked turtle; sex not known; sub-order Pleurodira, § 96 h exerction. || 48 h exerction.

In most of the animals studied, hippuric acid is the major metabolite, with benzoyl glucuronide appearing as a secondary metabolite and benzoic acid sometimes appearing in its free form (195). However, in two cases (ferret and fruit bat) benzoyl glucuronide predominated, while in three other cases (chicken, turtle, and gecko) ornithuric acid appeared as the major metabolite (195). A large volume of literature by many researchers confirms the findings of Bridges.

In man, the main site of benzoate detoxication is the liver (1068, 1474), where conjugation of the benzoate with glycine takes place by the following mechanism (1474):

benzoic acid + adenosine triphosphate - adenylbenzoate + pyrophosphate
adenylbenzoate + coenzyme A - benzoyl-coenzyme A + adenylic acid
benzoyl-coenzyme A + glycine - hippuric acid + coenzyme A

The formation of hippuric acid from sodium benzoate or benzoic acid has been found to be limited by the availability of glycine (21, 1064, 1474). The human body can produce about 0.62 g of glycine per hour (1056), which is enough to conjugate approximately 1.30 g of sodium benzoate or 1.5 g benzoic acid per hour (1068). When sodium benzoate or benzoic acid is administered to man, in the fasting state, in doses less than this it is excreted completely as hippuric acid (21, 195, 1064, 1474). If the dosage is greater than this, benzoyl glucuronide appears as a secondary metabolite (1064, 1474). The concurrent administration of glycine, or a substance which can be metabolized to form glycine, leads to an increased rate of hippuric acid formation (21, 680, 1064, 1474). Normal urinary excretion of hippuric acid in man is 1.0 - 2.5 g/day, which is equivalent to 0.8-2.0 g of sodium benzoate or 0.7-1.7 g of benzoic acid (1299). Quick found that the ingestion of sodium benzoate, when glycine is not a limiting factor, can increase this rate to approximately 46 g/day, calculated as sodium benzoate or 40 g/day, calculated as benzoic acid (1064). In a more recent study by Schachter, the maximum output of sodium benzoate in the form of hippuric acid was found to be 24 g/day and of benzoic acid 20 q/day (1166).

The elimination of sodium benzoate or benzoic acid is rapid, with 75-100% appearing in the urine in the tirst six hours after administration (680, 1064). Even after repeated doses (2 g NaOBZ/day or 1.7 g of the acid/day for 8 days), two or three days was sufficient for total elimination (155).

Experiments on the distribution and elimination of $^{14}\text{C--benzoate}$ in the rat have shown no accumulation of sodium benzoate or benzoic acid in the body (750).

IV. The Effects on Enzymes and Other Biochemical Parameters

The utilization of glycine in the detoxication of benzoate results in a reduction of the glycine level of the body (319, 1227). Therefore, the ingestion of benzoic acid or its salts effects any body function or metabolic process in which glycine is involved. For example, the ingestion of sodium benzoate leads to reduction in creatine (183), glutamine (527),

urea (1215, 1319), and uric acid (1067, 1319) formation due to preferential synthesis of glycine.

Benzoáte accelerates the autoxidation of oxyhemoglobin (563, 671, 1383, 1463) and myoglobin (662) in vitro by interaction with the hemeprotein. It also promotes the formation of green pigments through the oxidative disintegration of hemoglobin (563).

The administration of benzoic acid or sodium benzoate shortens prothrombin time, probably through increasing the level of thromboplastin cofactor (566).

The oral administration of sodium benzoate to rats decreased the rate of fat absorption (609), and altered the metabolism of lipids and electrolytes (723). As a result, decreases in the growth rate, the level of liver phospholipids and the potassium level of skeletal muscle were observed (723).

In vitro studies of guinea pig liver slices have also shown sodium benzoate inhibition of the oxidation of fatty acids (643, 644).

Bosund found that in vitro benzoate uncouples the oxidative phosphorylation from respiration in mitochondria (164). However, in similar experiments, Brody found no such effect (197).

Benzoic acid or sodium benzoate inhibits amino acid oxidases (687, 688, 714), alpha-chymotrypsin (580), glutamic acid decarboxylase (549), glycine acyltransferase (184), catalase (509) and lactic dehydrogenase (53). The Kreb's cycle is affected by benzoate through inhibition of alpha-ketoglutaric dehydrogenase and succinic dehydrogenase (651).

Intragastric administration of sodium benzoate or benzoic acid increased the tyrosine-alpha-ketoglutarate transaminase level in the rat (1232). Inhibition of this increase by injected puromycin and actinomycin D suggest that this effect of benzoate occurs through a mechanism involving increases in protein and RNA synthesis (1232).

V. Drug Interaction

Aspirin, acetylsalicylic acid, is metabolized in the body to form salicylic acid, which is then detoxified by conjugation with glycine. Concurrent administration of sodium benzoate depletes the supply of available glycine and thus increases the concentration and persistence of salicylic acid (21, 673, 772). The result of this is an increase in the teratogenic action of aspirin (673), salicylic acid having been implicated as the causative agent. By eating 1000 g, or about 2 lbs, of the right kinds of food a human being might consume as much as 1 g of sodium benzoate in a day. Levy et al. (772) demonstrated almost total inhibition of salicyluric acid formation in man using 3.2 g of sodium benzoate or 2.7 g benzoic acid. Since the body's capacity for making salicyluric acid is limited (771) a smaller dose of benzoate might have a similar effect.

The effect of the simultaneous administration of sodium benzoate with procaine, lidocaine, cocaine, tetracaine, or dibucaine was studied in surface anesthesia (rabbit cornea) and infilterative anesthesia (rabbit tooth pulp). Sodium benzoate decreased the induction time and increased the potency and the duration of the effect of all the anesthetics to a significant extent (902).

The simultaneous administration of sodium benzoate with penicillin increases and prolongs the serum penicillin level by inhibition of renal excretion (143, 198, 1277). However, this occurs only with severe restriction of fluid and salt intake (143, 198, 1444).

In a toxicity study on rats and mice, the toxic effects of orally administered sodium benzeate and sodium bisulphite, when administered in combination, appeared to be synergistic or additive to some extent (1218).

It has also been reported that sodium chloride has a considerable synergistic effect with sodium benzoate (246).

Bubnoff et al. reported that orally administered benzoic acid exhibits an anticonvulsive effect in rats with cocaine-induced cramps (208).

VI. Consumer Exposure Information

Benzoic acid is most suitable for toods and beverages which naturally are in the pH range below 4.0 or 4.5 or can be brought into that range by acid addition. It is used in carbonated and still beverages, syrups, fruit salads, icings, jams, jellies, preserves, salted margarine, mincemeat, pickles and relishes, pie and pastry fillings, prepared salads and fruit cocktails. Use levels range from 0.05% to 0.10% (246).

The use of sodium benzoate or benzolc acid as a preservative in food and beverages is limited by law to one part per thousand (246).

A total of 72,703 pounds of benzoic acid and 2,274,835 pounds of sodium benzoate were used in foods in the United States in 1970 (943).

BENZOIC ACID

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